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Comparative Study of Immune Status to Infectious Agents in Elderly Patients with Multiple Myeloma, Waldenstrom's Macroglobulinemia, and Monoclonal Gammopathy of Undetermined Significance[▽]

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Whereas patients with multiple myeloma (MM) have a well-documented susceptibility to infections, this has been less studied in other B-cell disorders, such as Waldenstrom's macroglobulinemia (WM) and monoclonal gammopathy of undetermined significance (MGUS). We investigated the humoral immunity to 24 different pathogens in elderly patients with MM ($n = 25$), WM ($n = 16$), and MGUS ($n = 18$) and in age-matched controls ($n = 20$). Antibody titers against pneumococci, staphylococcal alpha-toxin, tetanus and diphtheria toxoids, and varicella, mumps, and rubella viruses were most depressed in MM patients, next to lowest in WM and MGUS patients, and highest in the controls. In contrast, levels of antibodies specific for staphylococcal teichoic acid, *Moraxella catarrhalis*, candida, aspergillus, and measles virus were similarly decreased in MM and MGUS patients. Comparable titers in all study groups were seen against *Haemophilus influenzae* type b (Hib), borrelia, toxoplasma, and members of the herpesvirus family. Finally, a uniform lack of antibodies was noted against *Streptococcus pyogenes*, salmonella, yersinia, brucella, francisella, and herpes simplex virus type 2. To conclude, although MM patients displayed the most depressed humoral immunity, significantly decreased antibody levels were also evident in patients with WM and MGUS, particularly against *Staphylococcus aureus*, pneumococci, and varicella. Conversely, immunity was retained for Hib and certain herpesviruses in all study groups.

Patients with malignancies and dysfunctions of the B-cell lineage have impaired immunity and an increased risk of contracting severe infections. This is well documented in the case of multiple myeloma (MM), while Waldenstrom's macroglobulinemia (WM) and monoclonal gammopathy of undetermined significance (MGUS) have been less studied in this respect. Despite the use of prophylactic antimicrobial agents, infections remain a leading cause of morbidity and mortality in MM patients (7, 32). Bacterial infections predominate, in particular those caused by *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Haemophilus influenzae*, and *Escherichia coli* (7, 23, 29). However, the introduction of autologous stem cell transplantation and novel therapeutic agents, e.g., thalidomide, bortezomib, and lenalidomide, has led to a shift in the spectrum of infections in MM patients such that viral and fungal infections are increasingly diagnosed (1, 29). The highest risk of infection occurs within the first months after diagnosis of MM (32), especially in patients with renal failure (7, 29). Augustson et al. showed that 45% of early deaths in MM (within 60 days of diagnosis) were due to infections, mainly pneumonia and sepsis (5).

Information regarding which types of infections that tend to afflict patients with WM or MGUS is sparse. In a study of 217 WM patients, the second most frequent cause of death next to disease progression was infectious diseases (19% of deaths); again, sepsis and pneumonia predominated (15). An increased risk of bacteremia has previously been described for MGUS patients (19). Moreover, a recent nationwide Swedish study reported an excess mortality due to bacterial infections among MGUS patients, with a hazard ratio of 3.4 (27).

The B-cell dysfunction is more profound in MM than in WM and MGUS and features a reduction in specific antibodies as well as increased frequency of autoimmune B cells (30, 31). An important point is that these disorders affect mainly the elderly, in whom an age-related decline in immune functions is additionally seen, encompassing both the innate and the adaptive immune systems (17). As a consequence, the prevalence of bacterial urinary tract infections, pneumonia, and septicemia, as well as viral infections, such as influenza and herpes zoster, is higher in aging populations (17). Moreover, quantitative and functional defects in T cells and NK cells contribute to the immunodeficiency seen in patients with B-cell disorders and malignancies (30, 31, 32). As an example, MM, WM, and MGUS are all characterized by reduced numbers of $CD4^+$ T

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TABLE 1. Patient characteristics

Study group	No. of patients	Median age in yr (range)[a]	No. of females (%)	No. of patients with ongoing immuno modulatory therapy (%)	Median level of S-IgG in g/liter[b] (range)	No. of patients with hypogammaglobulinemia (%)	Median level of M-protein in g/liter (range)
Multiple myeloma	25	77 (62–88)	12 (48)	16 (64)	1.7 (0.1–8.0)	23 (92)[c]	26 (0.7–49)
Waldenstrom	16	77 (62–90)	9 (56)	1 (6)	7.0 (1.0–11)	7 (44)	16 (3.0–35)
MGUS	18	71 (60–83)	11 (61)	0 (0)	5.3 (0.3–11)	10 (56)	12 (0.5–28)
Control	20	69 (61–83)	11 (55)	0 (0)	11 (8.2–19)	0 (0)	0 (0–0)
All groups	79	75 (60–90)	43 (54)	17 (22)	NA[d]	NA	NA

[a] No statistically significant differences between the study groups. $P = 0.02$ for Waldenstrom versus controls.
[b] Reference level in serum is 6.1 to 14.9 g/liter. For IgG myeloma and IgG MGUS, S-IgG was calculated by subtracting the M-protein from total serum IgG.
[c] Statistically significant versus Waldenstrom, $P = 0.0002$; $P = 0.01$ versus MGUS.
[d] NA, not applicable.

cells (30, 31), with a concomitant impairment of cellular immunity.

Antigen-specific antibodies produced by B cells protect the host from extracellular bacterial infections through immune mechanisms, including neutralization, complement activation, opsonization, and in the case of intracellular pathogens, enhancement of cellular toxicity (28). The hypogammaglobulinemia that commonly occurs in primary as well as in secondary immunodeficiencies renders patients susceptible to infections caused by encapsulated bacteria, such as *S. pneumoniae* and *H. influenzae* (37). The immune defense active against primary viral infections is mainly cell mediated, while specific antibodies play an important role in preventing reinfection, often by viral neutralization (28).

Two previous studies have shown a higher incidence of infections in MM patients than in WM and MGUS patients (10, 13). However, to our knowledge, no comparative studies of antimicrobial immunity have been conducted in these patient groups. The aim of this study was to investigate the humoral immune status to common infectious agents in elderly patients with these B-cell disorders and presumed secondary immunodeficiency. Our intention was to compare these patient groups with respect to patterns of susceptibility to a panel of clinically relevant bacterial, viral, fungal, and protozoan pathogens while taking into account the natural age-dependent decrease in humoral immunity.

MATERIALS AND METHODS

Study population. Patients with MM, WM, and MGUS, age 60 years or more and attending the outpatient clinic of the Department of Hematology, Uddevalla Hospital, were recruited to the study from May 2008 to March 2009. The WHO criteria were used to establish the diagnoses (25). In order to achieve more comparable patient groups with respect to treatment-induced immunosuppression, patients who had undergone hematopoietic stem cell transplantation or were on high-dose conditioning chemotherapy were excluded. An age-matched control group without hematological disorders and from the same geographical area was recruited over the same period. All study participants were asked to fill in a questionnaire about previous immunizations (tetanus, diphtheria, pneumococci, *H. influenzae* type b, varicella), and ongoing medication was recorded. Written informed consent was obtained from all participants. The study was approved by the Regional Ethics Committee in Göteborg, Sweden.

Patient characteristics are presented in Table 1. Among the MM patients, 16 had IgG myeloma, eight IgA myeloma, and one Bence-Jones myeloma. The MGUS patients had monoclonal protein (M-protein) of the IgG isotype in nine cases, IgA in four, and IgM in three, and one patient had an undefined M-protein isotype. A biclonal gammopathy (IgG and IgA) was seen in one case. Ongoing immunomodulatory therapies in the MM group consisted of melphalan and prednisone in five cases, cyclophosphamide and dexamethasone in six, pulse steroids in three, thalidomide in two, and bortezomib in two. Only one of the WM patients was receiving immunomodulatory medication (fludarabine) at the time of the study. Three of the MM patients (12%), eight of the WM patients (50%), and all MGUS patients were treatment naïve.

Sampling of study persons. Peripheral blood (33 ml) was collected once from each study person, by venipuncture into Vacutainer tubes. Both EDTA-anticoagulated blood and whole blood were used. The EDTA-anticoagulated sample was directly analyzed at the Department of Clinical Chemistry, NU Hospital Organization, for routine hematological parameters (hemoglobin rates, white blood cell count, platelet count, and blood differential counts) using Abbott CellDyn 4000 (Abbott, Chicago, IL). Concentrations of IgA, IgG, and IgM in serum were measured through turbidimetry using Architect c8000 (Abbott). Serum levels of M-protein and free light chains were determined by agarose gel electrophoresis with immunofixation (Hydrasys LC, Sebia, Evry, France). For IgG myeloma and IgG MGUS, serum IgG was calculated by subtracting the M-protein from total serum IgG. Remaining serum samples were stored at −20°C until analyzed.

Serological analyses. Thawed patient sera were analyzed for IgG antibody titers to microbes by validated methods at the Departments of Clinical Bacteriology and Virology, Sahlgrenska University Hospital. Commercial kits, manufacturers, and previously published in-house methods are presented in Table 2. The enzyme-linked immunosorbent assay (ELISA) employed for analysis of anti-herpes simplex virus (HSV) IgG detects antibodies to both HSV type 1 (HSV1) and type 2 and is used in combination with an ELISA specific for HSV2.

Antibody titers against *Moraxella catarrhalis* were measured by ELISA. Briefly, *M. catarrhalis* strain Bc5 was cultured in brain heart infusion (BHI) broth overnight and frozen. Microtiter plates were coated with freeze-thawed lysates of *M. catarrhalis* diluted in Tris-buffered saline, pH 9.6, for 2 h. Plates were blocked for 1 h in phosphate-buffered saline (PBS) containing 1.5% bovine serum albumin (BSA) and 0.05% Tween 20, and serum samples were added in duplicate at 2-fold dilutions starting at 1:20 and ending at 1:2,560. After incubation for 1 h, plates were washed and incubated with alkaline phosphatase-conjugated secondary goat anti-human IgG polyclonal antibody (pAb) (Jackson ImmunoResearch Europe, Newmarket, United Kingdom) for 1 h followed by an additional wash. Plates were developed using phosphatase substrate (Sigma-Aldrich, St. Louis, MO) dissolved in diethanolamine buffer, pH 9.8. All incubations were performed at room temperature.

For the Epstein-Barr virus (EBV) indirect immunofluorescence (IF) assay, latently infected P3HR1 cells expressing EBV capsid antigen (VCA) were used. Antibodies to human herpesvirus type 6 (HHV6) were determined by IF using latently infected cells expressing HHV6 antigens. Whole-virus antigen prepared from Edmonton morbillivirus (reference strain)-infected green monkey kidney (GMK) cells was used for the measles virus ELISA. Anti-parotitis virus IgG was detected by IF using mumps virus-infected GMK cells. Serum samples were diluted 1:100 and titrated to 1:6,400 for analysis of IgG antibodies to cytomegalovirus (CMV), HSV, HSV2, varicella-zoster virus (VZV), morbillivirus, and *Toxoplasma gondii*, diluted from 1:20 to 1:2,500 for the analysis of anti-HHV6 IgG and from 1:4 to 1:64 for anti-parotitis virus IgG determinations. The assay used for anti-EBV IgG measurements is qualitative and tests samples at two dilutions, 1:16 and 1:32. All serological assays, including in-house assays, were done at accredited laboratories (SWEDAC, Swedish Board for Accreditation and Conformity Assessment), whose performance is regularly tested by participation in the quality assessment schemes of United Kingdom NEQAS (United

TABLE 2. Immune assays for determination of antibody titers to bacterial, fungal, viral, and protozoan antigens

Agent	Method	Manufacturer	Reference
Tetanus toxoid	ELISA	Euroimmun, Lübeck, Germany	
Diphtheria toxoid	ELISA	Euroimmun	
Staphylococcus aureus antigens teichoic acid and alpha-toxin	ELISA	PhPlate Microplate Techniques, Stockholm, Sweden	
Streptococcus pyogenes antigen streptolysin-O	Particle agglutination test (SERODIA-ASO)	Fujirebio, Tokyo, Japan	
Streptococcus pyogenes antigen DNase B	Neutralization test	PhPlate Microplate Techniques	
Pneumococci	ELISA	The Binding Site Group, Birmingham, UK	
Haemophilus influenzae type b (Hib)	ELISA	The Binding Site Group	
Moraxella catarrhalis	ELISA[a]	In-house, Skåne University Hospital, Malmö, Sweden[b]	
Extended Widal reaction for *Salmonella* BO, *Salmonella* DO, *Brucella abortus*, *Francisella tularensis*, *Yersinia enterocolitica* serotypes 03 and 09	Agglutination test	In-house[b] antigens from Reagensia, Solna, Sweden; samples diluted from 1:40 to 1:5,120	16
Borrelia burgdorferi	Chemiluminescence (Liaison Borrelia)	DiaSorin, Saluggia, Italy	
Candida albicans	ELISA	Institut Virion/Serion, Würzburg, Germany	
Aspergillus fumigatus	ELISA	Institut Virion/Serion	
Cytomegalovirus (CMV)	ELISA	In-house[c]	3, 11
Epstein-Barr virus (EBV)	Immunofluorescence[a]	In-house[c]	
Herpes simplex virus (HSV)	ELISA	In-house[c]	26
Herpes simplex virus type 2 (HSV2)	ELISA	In-house[c]	36
Varicella-zoster virus (VZV)	ELISA	In-house[c]	14
Human herpesvirus type 6 (HHV6)	Immunofluorescence[a]	In-house[c]	
Measles virus (morbilli)	ELISA[a]	In-house[c]	
Mumps virus (parotitis)	Immunofluorescence[a]	In-house[c]	
Rubella virus	Chemiluminescence microparticle immunoassay (CMIA)	Abbott Laboratories, Abbott Park, IL	
Toxoplasma gondii	ELISA	In-house[c]	3

[a] See Materials and Methods.
[b] Department of Clinical Bacteriology.
[c] Department of Clinical Virology.

Kingdom National External Quality Assessment Service for Microbiology) and Equalis (External Quality Assessment for Clinical Laboratories in Sweden).

Statistical analyses. The nonparametric two-tailed Mann-Whitney test was used to determine statistically significant differences between study groups. For statistical calculations of samples in which antibody titers were below the detection threshold, the cutoff level for the assay in question was divided by two. Statistically significant rates of seropositivity were determined using Fisher's exact test. Univariate analyses were performed using GraphPad Prism 5.0 software (GraphPad, San Diego, CA). A significance level of P values of <0.01 was chosen to compensate for multiple analyses.

RESULTS

The study population had a comparable proportion of women and men and no significant age differences between the study groups (Table 1). Hypogammaglobulinemia was found in a majority of MM patients (92%), as opposed to in 44% and 56% of the WM and MGUS patients, respectively (Table 1).

When comparing the serum IgG antibody levels directed against the 24 tested infectious agents among the study groups, four patterns of humoral immunity could be discerned. The first pattern featured stepwise antibody titers, with the lowest seen among MM patients, next lowest among WM and MGUS patients, and highest in the control group. This pattern was evident for the following pathogens: *S. aureus* regarding antibodies to alpha-toxin, pneumococci, tetanus, diphtheria, and VZV (Fig. 1). Median levels of anti-alpha-toxin (*S. aureus*) antibodies were 3-fold higher in the control group than in the MM group ($P = 0.0004$) (Fig. 1A). Levels of anti-pneumococcal antibodies were 15-fold higher in the controls than in the MM group ($P < 0.0001$) and 5-fold higher in controls than in the WM group ($P = 0.002$) (Fig. 1B). A significant difference was seen also between the MM and the MGUS groups ($P < 0.0001$). Twelve persons, three from each study group, reported previous pneumococcal vaccination. No differences in titers were found when comparing these to the unvaccinated part of each study group. Previous tetanus immunization was reported by 68% of the study individuals, and diphtheria immunization was reported by 35%. Protective antibody levels to tetanus ranged from 8% among MM patients to 65% of healthy controls ($P < 0.0001$) (Fig. 1C). In the case of diphtheria, no study person exhibited long-term immunity (>1.0 IU/ml) (Fig. 1C). Short-term immunity requiring vaccine booster ranged from 4% in the MM group to 35% in the control group ($P = 0.01$).

Seropositivity to VZV was seen in 68% of MM patients, 75% of WM patients, 94% of MGUS patients, and 100% of healthy controls ($P = 0.006$ for MM versus the control group), with an 8-fold-higher median antibody level in the control group than in the MM group (Fig. 1D). Five individuals reported previous immunization to VZV, all of whom had high antibody levels. The analyses of mumps virus and rubella virus

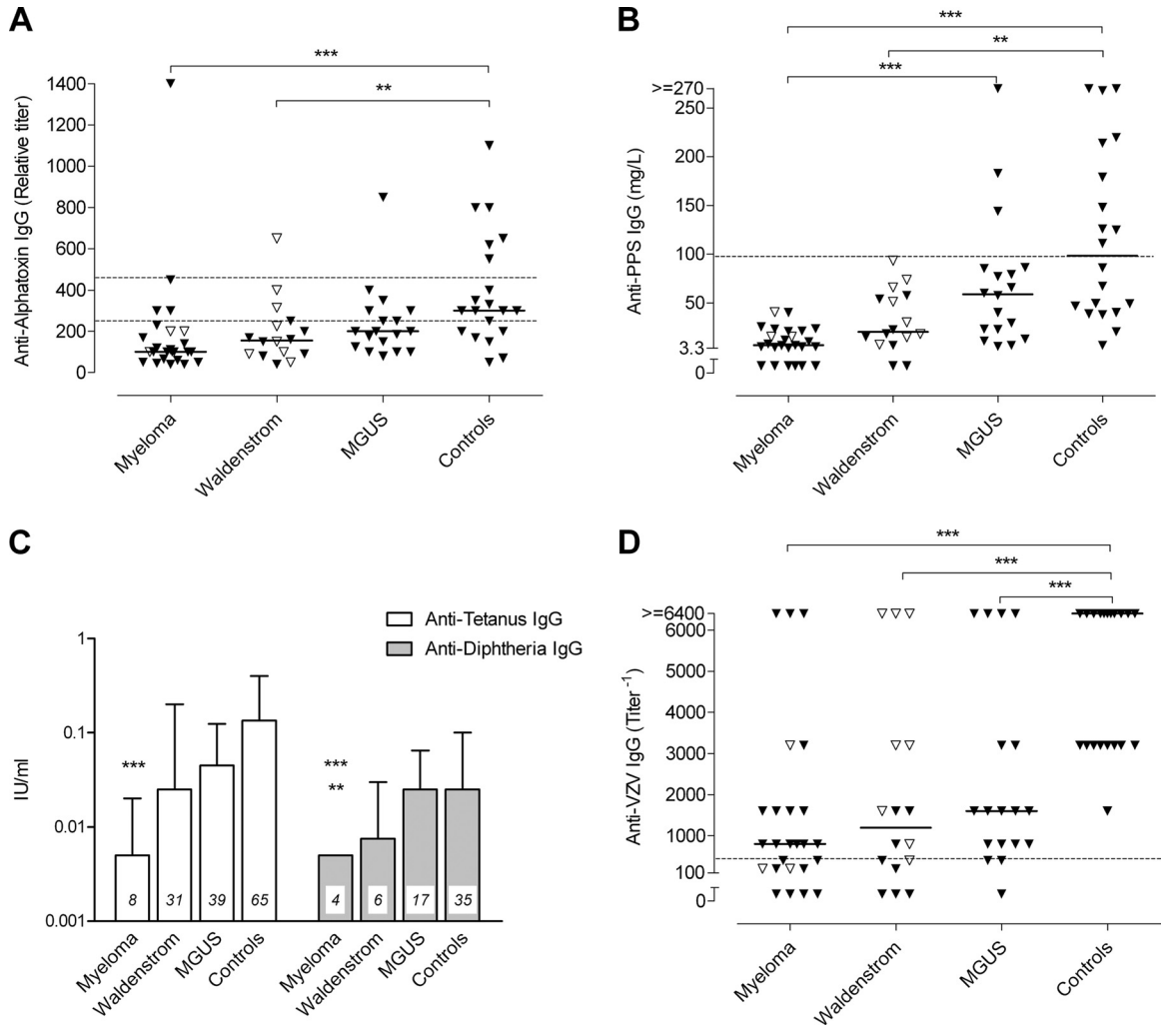

FIG. 1. Stepwise antibody titer pattern. (A) Anti-staphylococcal IgG antibody titers to alpha-toxin. Each triangle represents one individual. Open triangles represent treatment-naïve MM patients ($n = 3$) and WM patients ($n = 8$). Horizontal bars denote the median value for each study group. The dotted lines indicate age-stratified population medians; the upper line for those age >75 years, the lower for the 60-to-75-year bracket. (B) Anti-pneumococcal IgG antibody titers to pneumococcal polysaccharide (PPS). The dotted line indicates the control group median antibody level. (C) Anti-tetanus and anti-diphtheria toxoid IgG. The bars and error bars indicate group median titers and interquartile ranges. Numbers enclosed in the bars represent percentage with protective immunity, in the case of diphtheria short-term immunity. **, $P < 0.01$, versus MGUS; ***, $P < 0.001$, versus control group. (D) Anti-varicella-zoster virus (VZV) IgG. The dotted line indicates the cutoff level for protective immunity (400). ***, $P < 0.001$.

antibodies also revealed a stepwise increase from the MM group to healthy controls (not shown). For mumps virus, a 4-fold-higher median antibody level was seen among the controls compared to that of the MM group ($P = 0.006$), and for rubella the difference was 2-fold (not significant [NS]). However, seroprevalence was high: 84% for mumps, 81% for rubella in the study population as a total, and above 70% in each of the study groups.

In the second pattern of humoral immunity noted, antibody titers were more depressed in MM and MGUS patients than in the WM and control groups. This was seen for the second staphylococcal antigen, teichoic acid, *M. catarrhalis*, candida, and aspergillus (Fig. 2), as well as for measles virus (not shown). As was earlier described for mumps and rubella, the seroprevalence to measles was high, 96% overall, with only 3/79 persons deemed to be seronegative. Median levels of teichoic acid (*S. aureus*) antibodies were 4-fold higher in the control group than in the MM group ($P < 0.0001$) (Fig. 2A). Robust moraxella antibody levels of comparable magnitude were seen among the age-matched controls and WM patients and were clearly higher than those among the MGUS and MM patient groups (Fig. 2B). For moraxella as well as for staphylococcal teichoic acid, significant differences in antibody levels were found also among the patient groups (Fig. 2A and B). Regarding antibodies directed against candida and aspergillus, once again, the lowest titers were detected in the MM and MGUS groups (Fig. 2C and D). Borderline and positive aspergillus titers were more frequent among the WM patients and controls than in the MM and MGUS groups (Fig. 2D).

The third pattern of antimicrobial humoral immunity was characterized by measurable and comparable levels of antibody titers among all study groups. This was seen for *H. influ-*

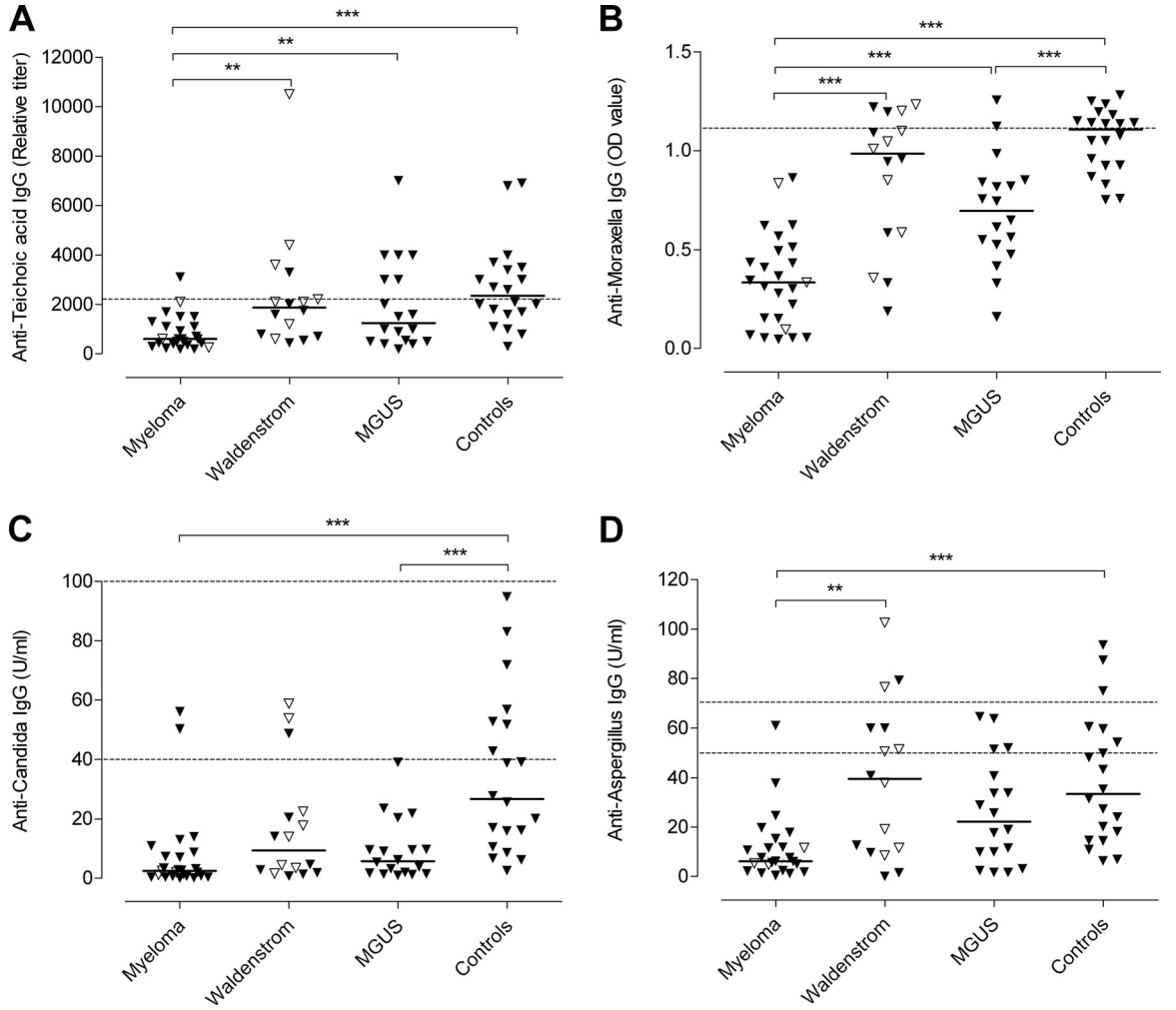

FIG. 2. Antibody titers most depressed in MM and MGUS patients. Each triangle represents one individual. Open triangles represent treatment-naïve MM patients ($n = 3$) and WM patients ($n = 8$). Horizontal bars denote the median value for each study group. (A) Anti-staphylococcal IgG antibody titers to teichoic acid. The dotted line indicates a population median for those age ≥60 years. (B) Anti-moraxella IgG (OD value) measured in serum samples diluted 1:80. The dotted line indicates the control group median OD level. (C) Anti-candida IgG. Titers between the dotted lines (40 to 100) are considered borderline, titers <40 are considered negative, and titers >100 are considered positive with respect to ongoing infection. (D) Anti-aspergillus IgG. Titers between the dotted lines (50 to 70) are considered borderline, titers <50 are considered negative, and titers >70 are considered positive. **, $P < 0.01$; ***, $P < 0.001$.

enzae type b (Hib), borrelia, the herpes group viruses (except for VZV and HSV2), and toxoplasma (Fig. 3). A majority of the study participants (80% of MM patients, 88% of WM patients, and 100% of MGUS patients and healthy controls) had protective antibody concentrations against Hib even though no one had been vaccinated (Fig. 3A). An unexpectedly large number of persons were seropositive to borrelia (27%), many of whom displayed high antibody titers (Fig. 3B). High antibody titers without significant differences between the study groups were seen against the herpesviruses HSV1 (Fig. 3C), CMV, and EBV (not shown), with a total seroprevalence among our study participants of 86% for HSV1, 75% for CMV, and 84% for EBV. HHV6 and toxoplasma antibody titers did not differ significantly between the study groups, but fewer MM patients were seropositive than in the other groups (data not shown).

The last pattern of humoral immunity to microbes consisted of an absence of antibody titers in all study groups, including the control group. Regarding the streptococcal antigens streptolysin O and DNase B, a few individuals, from all study groups, displayed measurable but low antibody titers. Concerning antibodies against enteric pathogens, only one person, from the MGUS group, had a low antibody titer (1:40) to *Salmonella* BO and DO; all other participants were seronegative, which was also the case for *Yersinia enterocolitica* serotypes 03 and 09. No study person had measurable antibody titers to *Brucella abortus* or *Francisella tularensis*. Four participants (5%) were seropositive to HSV2, two MM patients and two controls.

Treatment-naïve MM patients ($n = 3$) and WM patients ($n = 8$) had a tendency toward higher antibody titers than patients with previous and/or ongoing chemotherapy or immunomodulatory treatment (Fig. 1 to 3). Since these subgroups were small, no statistical analyses were performed.

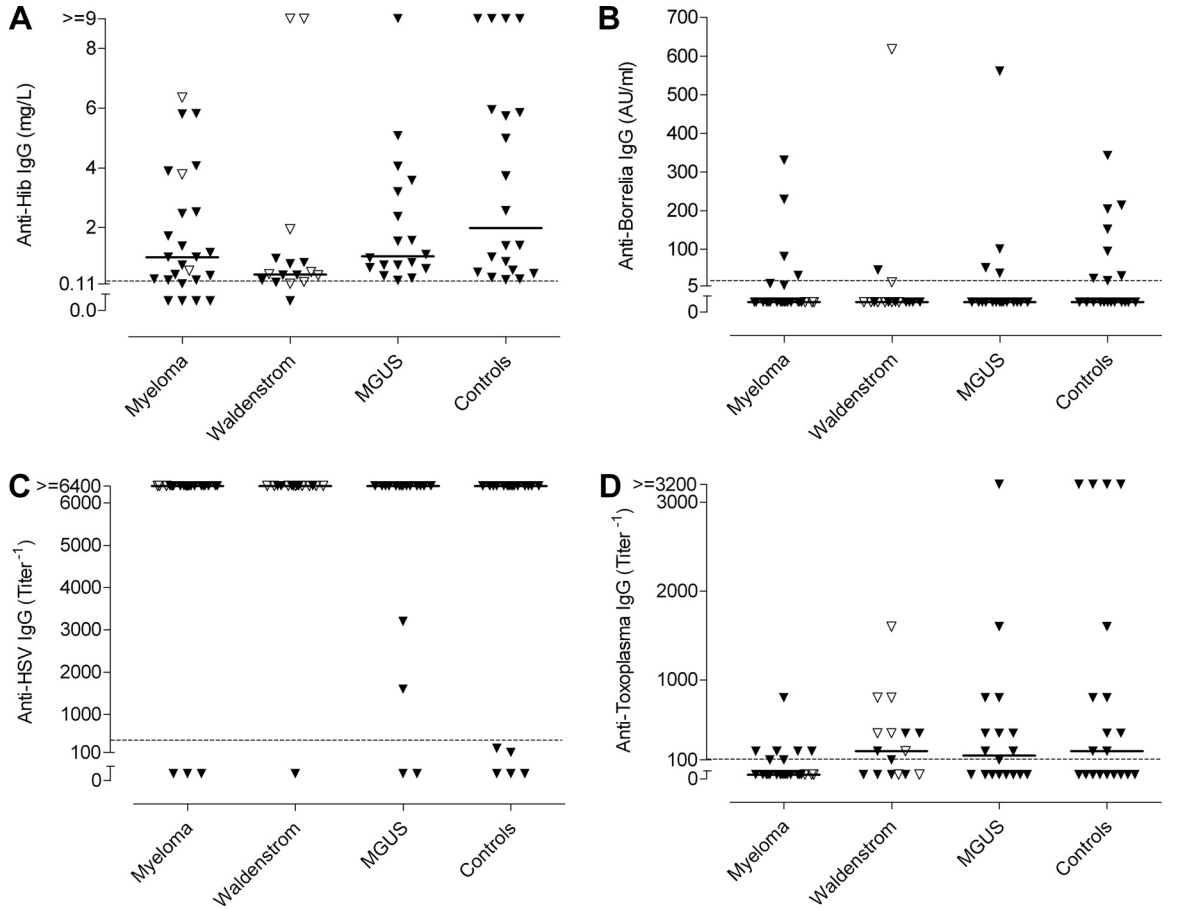

FIG. 3. Comparable antibody titers among all study groups. Each triangle represents one individual. Open triangles represent treatment-naïve MM patients ($n = 3$) and WM patients ($n = 8$). Horizontal bars denote the median value for each study group. (A) Anti-*Haemophilus* (Hib) IgG. The dotted line (0.15) represents a minimum protective antibody level. (B) Anti-borrelia IgG; (C) anti-herpes simplex virus (HSV) IgG; (D) anti-toxoplasma IgG. In panels B to D, the dotted lines indicate the levels of seropositivity; 15 AU/ml (B), 400 AU/ml (C), 100 AU/ml (D).

DISCUSSION

As expected, patients with MM had the most suppressed humoral immunity toward the studied pathogens. For a number of pathogens, we found significant differences not only for MM patients versus healthy controls but also between MM and WM and/or MGUS groups. This is consistent with previous reports documenting a higher incidence of serious infections in MM patients than in WM and MGUS patients. One study estimated that MM patients were 10 times more infection prone than WM patients and 5 times more at risk than MGUS patients (10). Such figures will vary depending upon the age of the selected patients and the degree of treatment-induced immunosuppression. We chose to focus on elderly patients, since they are the group most often afflicted by B-cell malignancies and dysfunctions. Moreover, patients who had undergone high-dose chemotherapy and/or autologous stem cell transplantation were excluded in order to avoid a too-drastic imbalance between the study groups. These types of patients have been less studied than transplant recipients. Nevertheless, although higher infection rates among MM patients are correlated with active disease and ongoing chemotherapy (7, 20), a continuous increased risk of infection has been shown in patients with plateau phase myeloma and was typically associated with poor IgG antibody production (20). The depressed humoral immunity seen in the three patient groups studied is partly a function of the subnormal serum IgG levels displayed by a large proportion of the patients. Moreover, there was a tendency toward higher antibody titers among the treatment-naïve MM and WM patients than among those with ongoing and/or previous immunomodulatory treatment. However, these subgroups were small, and further studies are needed to confirm these findings.

The MGUS group displayed surprisingly low antibody levels to a number of pathogens, notably staphylococcal antigens, moraxella, VZV, and fungal antigens. This certainly correlates to the facts that our patient cohort had a high rate of hypogammaglobulinemia (56%), high levels of M-protein (median, 12 g/liter), and a relatively high median age (71 years). Previous studies have shown increased mortality from bacterial infections as well as a higher risk of bacteremia in MGUS patients than in age-matched controls (18, 19, 27). On the other hand, a recent retrospective study

by Bida et al. could not confirm an association between MGUS and infections, except for those caused by mycobacteria (6). MGUS patients are a heterogeneous group, covering a spectrum from healthy persons with stable and low M-protein levels to patients on the border to those with malignant transformation. Hence, their degree of immunosuppression is likely to vary greatly. Also, MGUS patients have a high incidence of underlying diseases, such as autoimmune disorders and nonhematological malignancies, that might have been the cause for examination of the patient and detection of the M-protein in the first place (6, 18, 27). Such comorbidities may contribute additionally to the increased susceptibility to infections displayed by MGUS patients.

A decline in specific antibody titers has been described with increasing age (17), adding to the effects of the B-cell disease. We cannot exclude that age differences among our study groups, although not statistically significant, may have influenced the results, in particular those pertaining to comparisons of the WM group with the control group. It is evident that the pattern of infections seen in healthy elderly individuals overlaps with that described in individuals with immunodeficiency secondary to B-cell malignancies and dysfunctions. In both cases, bacterial infections are predominant, particularly those caused by *S. pneumoniae*, *S. aureus*, and Gram-negative rods (7, 17, 23).

Four antibody patterns emerged from our serological analyses. These included a stepwise antibody titer increase from MM patients to healthy controls (e.g., pneumococci, staphylococcal alpha-toxin, VZV), more depressed antibody titers in the MM and MGUS groups relative to those of the WM and control groups (e.g., staphylococcal teichoic acid, candida, aspergillus), measurable and comparable levels of antibody titers among all study groups (e.g., Hib, several herpesviruses), and absence of antibody titers in all study groups (e.g., streptococcal antigens, HSV2, various enteropathogenic bacteria). The last pattern is likely to reflect lack of exposure to pathogens such as HSV2 and enteropathogenic bacteria rather than waning antibody titers. Background anti-streptococcal antibody titers are known to be low in adult populations of developed countries (35), which was confirmed in our study.

Low baseline antibody titers to pneumococci have previously been found in MM patients (20, 33) and have been associated with increased risk of serious infection (20). In our study, significantly lower titers were demonstrated in both MM and WM groups than in healthy controls, which fits with the documented susceptibility of these patients to pneumococcal infections (7, 23, 29). It also highlights the issue of vaccination in these patient groups; however, previous studies on pneumococcal vaccination in MM patients have had discouraging results (20, 33). Our control group displayed a high median titer against pneumococci in comparison to adult titers reported in a previous study (34). This study population was indeed younger than ours (20 to 61 years of age). Our results probably reflect a high prevalence of pneumococcal infections in the elderly (17) resulting in antibody boosting. However, there is no consensus regarding what titers of pneumococcal antibodies actually confer protective immunity in adults.

We also found noticeably lowered antibody levels to staphylococcal antigens in all three patient groups compared to those of our control group and a Swedish population median given for the assay in use. Low initial antibody levels in patients with invasive *S. aureus* infections have been shown to correlate with increased mortality and complicated bacteremia (24). High mortality due to *S. aureus* bacteremia has been previously reported for MM patients (12). In contrast, antibody titers to Hib did not differ significantly among our study groups, reflected by the finding that between 80 and 100% had antibody titers at or above the generally accepted minimum level protective against invasive Hib infection (0.15 mg/liter), which is comparable to protective rates in a healthy adult population (22). Despite this, Hib infections have been frequently reported in MM patients (7, 23). The MM patient group has previously been reported to respond to Hib vaccination, which is in contrast to the poor antibody response seen upon immunization against pneumococci (33). Immunity to tetanus and diphtheria was poor for all patient groups, and revaccination should be considered. A surprisingly large proportion of the controls also lacked protective antibodies, reflecting waning antibody titers but also revealing a high number of unvaccinated persons.

Viral antibody levels are known to be stable over time (2), which was confirmed in our study. High and comparable seroprevalence rates were observed among all the study groups for most of the viral pathogens assayed. An important exception was varicella: whereas the seroprevalence in the MGUS and control groups reached normal adult levels, one-fourth of our WM patients and almost one-third of the MM patients were seronegative, representing a loss of humoral immunity. VZV IgG antibodies confer protection by neutralizing the virus at sites of inoculation upon reexposure (4). Like all herpesviruses, VZV establishes a latent infection with the possibility of subclinical as well as symptomatic reactivation. Herpes zoster is well known in elderly individuals (17) and has been increasingly described in MM patients, not least among those treated with bortezomib (9, 29). However, VZV reactivation has been attributed to diminished cell-mediated immunity rather than to loss of antibodies (4, 9). It is possible that the patients with the lowest antibody titers in our cohort also had impaired T-cell function as a consequence both of their disease and of the given treatment; however, this needs to be confirmed in future studies. Our findings reinforce the need of prophylactic acyclovir in these patient groups, and with the development of an inactivated VZV vaccine, immunization may become a future option (21). Regarding HSV1 and CMV, surprisingly high seroprevalence figures were observed, with a tendency toward higher titers in the patient groups than in the healthy controls. This could be attributable to a higher number of reactivations of the viruses in the immunocompromised hosts resulting in boosting of antibody titers.

Antibody titers to the fungi examined, candida and aspergillus, were markedly lower in the patient groups than in the controls, foremost in MM but also in MGUS patients. It should be kept in mind that the candida IgG assay employed is designed to catch patients with ongoing infection, and the cutoff level has been set to ensure that 90% of healthy blood

donors test negative or borderline. Fungal infections in patients with B-cell malignancies are seen primarily following prolonged chemotherapy-induced neutropenia, high-dose corticosteroid treatment regimens, and stem cell transplantation (29). There is evidence of the protective potential of anti-candida antibodies to disseminated disease (8), and an increased susceptibility to candida is a possibility among our patients. The role of humoral immunity in the protection against aspergillus remains to be elucidated.

Evaluation of antibody levels and their relationship to antimicrobial immunity and susceptibility to infection is complicated since the protective nature of the antibodies, the longevity of the antibody response, and the specificity of the antibody tests vary greatly for different pathogens. While positive antibody titers in some cases, such as for streptococcal and fungal antigens, correlate with ongoing or recent infection, in other cases, such as HSV and CMV, the levels remain stable over time, presumably lifelong. The definition of protection also varies for different pathogens. In the case of Hib, a suggested antibody cutoff level refers to protection against invasive disease rather than complete protection against reinfection. The opposite, i.e., positive antibody titers defining a lifelong protection against the particular microbe, is the case for mumps, parotitis, and rubella.

This study aimed at describing a background antibody pattern to common microbes in patients with a presumed secondary immunodeficiency due to a B-cell malignancy or dysfunction and at defining possible risk pathogens. Our findings indicate an increased infectious susceptibility primarily in MM but also in WM and not least in MGUS patients. The most profound decrease in humoral immunity was seen for pneumococci, *S. aureus*, and VZV, but also for fungi, such as candida and aspergillus. In times of increasing antibiotic resistance, the identification of patients at high risk of developing severe infections, i.e., patients who would actually benefit from antibiotic prophylaxis or other preventive measures, such as immunizations, is of major importance. In the future, we would like to investigate T-cell responsiveness to microbes among these patient categories, as well as explore their innate immune function.

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